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Frailty in mouse ageing

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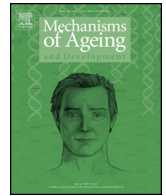
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Frailty in mouse ageing: A conceptual approach

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ABSTRACT

Human life expectancy has increased dramatically in the last century and as a result also the prevalence of a variety of age-related diseases and syndromes. One such syndrome is frailty, which is defined as a combination of organ dysfunctions leading to increased vulnerability to adverse health outcomes. In humans, frailty is associated with various biomarkers of ageing and predicts relevant outcomes such as responses to therapies and progression of health status and mortality. Moreover, it is relatively easy to assess. To foster translation of mechanistic understanding of the ageing process and, importantly, of interventions that may extend healthy lifespan, frailty scales have been reverse translated into mice in recent years. We will review these approaches with a view to identify what is known and what is not known at present about their validity, reproducibility and reliability with a focus on the potential for further improvement.

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1. Frailty in humans: definitions and operationalisations

Over the last 170 years, human life expectancy has been constantly increasing by 2–3 months every year without any indication of a slow-down (WHO, global health and aging, 2015). Historically, there have been different reasons for this increase, but presently, the main cause is probably improved management of chronic, age-associated diseases and disabilities. In other words, more and more people live longer but they suffer from an increasing number of chronic conditions, often resulting in decreased capability to perform their daily activities and they are frail.

1.1. Lifespan, healthspan and longevity trap

The medium life expectancy (lifespan for short) in EU member states for a 50 year old person increased by 1.2 years for men and 1.1 years for women between 2005 and 2010 (Fouweather et al., 2015) while healthy life expectancy, also called “healthspan” (Van Norman, 1995) increased only by 0.5 and 0.4 years, respectively, over the same time frame. Healthspan is defined as the length of time in one’s life where one is in optimal health (Van Norman, 1995) and inequalities between countries are presently much larger (about 3–4 fold) compared to lifespan. In a significant number of European countries, healthspan did in fact decrease between 2005 and 2010 (Global Health and Aging, 2015; Anon, 2016). In the UK, 85 year olds suffer from 5 (women) or 4 (men) chronic diseases on average at the same time (Collerton et al., 2009). The same trends exist worldwide, both in developed and low-income countries. Thus, a ‘longevity trap’ is becoming evident, inflicted by continuously increasing life expectancy together with stagnating healthy and disability-free periods. It has long been recognized that this situation urgently requires practicable and efficient counteraction that efficiently slows down the inevitable yet adaptable ageing process, thereby postponing the onset of age-related disease and disability.

1.2. Frailty as clinical syndrome of accelerated ageing

The concept of frailty has been developed to describe the conditions of aged people as physically weak with increased vulnerability to adverse health outcomes (Howlett, 2015) and is considered to be associated with a major loss of capacity to maintain tissue homeostasis and regeneration. Since frailty affects various parameters required for healthy living it negatively impacts healthspan. It is characterized by a state of age-related biological vulnerability to stressors and decreased physiological reserves with alterations in energy metabolism, decreased skeletal muscle mass and quality, and altered hormonal and inflammatory functions [reviewed by Mohler et al., 2014]. Recent consensus defined frailty as “a medical syndrome with multiple causes and contributors that is characterized by diminished strength and endurance, and reduced physiologic function that increase an individual’s vulnerability for developing increased dependency and/or death” (Morley et al., 2013). Importantly, the definition of frailty as a clinical syndrome rather than just an intuitive description of the ‘biologically oldest’ which specifies this state as a possible endpoint for interventions aimed at expanding healthspan. It is important to state that frailty is a plastic condition that can deteriorate but also revert over time. However, full-blown frailty marks a precipitous decline in overall

Table 1

Comparison of human frailty assessment tools proposed by Fried and Rockwood.

Fried’s frailty Phenotype ^a	Rockwood’s Frailty Index ^b
Clinical Syndrome 5 Phenotypic criteria	Deficit Accumulation Comprehensive Geriatric assessment (up to 70 parameters)
Associated with physical features Predictive for healthspan	Associated with biological markers Frailty is not differentiated from disability and co-morbidity
No cognitive parameter assessment Low inter-rater reproducibility	Includes cognitive assessments High inter-rater reproducibility

^a Ref. Fried et al., 2001.

^b Ref. Rockwood et al., 2005.

health with decreasing likelihood of recovery (Fried et al., 2001; Walston et al., 2006).

1.3. Frailty scales: Fried’s frailty phenotype and Rockwood index

A number of frailty scales have been developed to operationalise this concept [reviewed by Mohler et al., 2014 and Seldeen et al., 2015]. While all scales are able to predict mortality, sensitivity and specificity regarding the classification of individuals as frail/non-frail are at least partly different between the scoring systems (Theou et al., 2013; Ravindrarajah et al., 2013; Collerton et al., 2012). The two major frailty models that have been more extensively validated in the literature are the “Fried frailty phenotype” and the “Rockwood frailty index”. Fried’s frailty phenotype defines frailty as a distinct clinical syndrome meeting three or more of five phenotypic criteria: weakness, slowness, low level of physical activity, self-reported exhaustion, and unintentional weight loss (Fried et al., 2001). In contrast, Rockwood frailty index defined frailty originally as cumulative deficits identified in a comprehensive geriatric assessment (Rockwood et al., 2005). Deficiencies concerning more than 70 parameters relevant to everyday activities, also comprising physiological problems, mental capabilities, concomitant features of co-morbidities etc. have been included in the construction of the Rockwood frailty index. Major differences between the Fried and Rockwood scales are shown in Table 1. Even though multi-deficiency and multi-morbidity are an essential part of the frailty index, specific number and types of deficiencies diagnosed have only a minor impact on the categorization of frailty (Song et al., 2010). Moreover, frailty scales are closely associated with a wide range of markers of biological age, especially pro-inflammatory cytokine levels (Walston et al., 2006; Collerton et al., 2012; Leng et al., 2007). In fact, it is possible to use the same deficit concept to construct a frailty index based on biological markers of ageing (Mitnitski et al., 2015), supporting the view of frailty as exaggerated ageing.

2. Frailty measures in mice

There is a wide consensus in the ageing biology field that the time has come to apply the current understanding of the ageing process for distinct interventions in order to improve healthspan in humans. Mice are the most commonly used mammalian models in ageing research because of their relative ease of genetic manipulation, low cost and short lifespan (1–3 years depending on strain) (Yuan et al., 2009; Yuan et al., 2011 January 01). Moreover, many

studies have shown similarities in ageing processes between mice and humans (Grabner et al., 2013; Parks et al., 2012; Whitehead et al., 2014; Barreto et al., 2010) even though there are also significant differences to humans which limits specific applications. Important among these are the facts that mice exhibit higher regenerative capacities, muscle mass only minimally declines as mice age, mice have high telomerase activity in many organs, and mice are able to synthesize vitamin C (Vanhooren and Libert, 2013). Moreover, age-related disease spectra are very different in mice and humans, with cancer being prevalent in mice and major neurologic decline, cardiovascular disease and type 2 diabetes among others being much less prevalent in old mice than humans. Finally, laboratory mice are typically maintained as inbred strains, which is incomparable to humans and complicates or might even exclude comparative studies of genome-environment interactions. On the other hand, mouse breeding technology allows researchers to reduce biological variation as a source of experimental noise and also allows the exploitation of strain and cohort differences as a tool in ageing research (see for instance (Miwa et al., 2014)). Large scale longitudinal and cross-sectional studies assessing not only lifespan but also neuromuscular, kidney and heart function, hematology, hormone levels, immunological parameters, body composition, bone density, necropsy, pathology and apoptosis (Sundberg et al., 2011) are available for a wide range of inbred mouse strains in the Mouse Phenome Database (<http://phenome.jax.org>).

However, in many ageing studies, the focus has been on extension of lifespan in model organisms as a simple single criterion for efficiency of an intervention to delay ageing. To develop and validate interventions with potential to improve healthspan in humans with much higher impact for patients, this focus is no longer sufficient. Two overlapping approaches have been taken, namely i) the development of measures of healthspan in mice and ii) the reverse translation of the frailty concept. The state-of-the-art with regard to healthspan indices in mice has very recently been critically and exhaustively summarized by Richardson and colleagues (Richardson et al., 2016). They recommend the assessment of overall health in several domains based on the acceptance of the full complexity of so-called ‘anti-ageing’ interventions which will typically be segmental, i.e. delay degeneration or even improve health in some domains but result in worse outcomes in others. To standardize methodology, Richardson et al., 2016 compiled a comprehensive list of healthspan assays, providing an excellent tool for future intervention studies. In the present review, we will focus on attempts to reverse translate the frailty concept into mice. In recent years, both the Fried frailty phenotype and the Rockwood frailty index have been translated into mice (see Table 1). These efforts have been reviewed by (Howlett, 2015; Seldeen et al., 2015) but we feel that the importance of the topic warrants an update.

2.1. Fried-based mouse frailty phenotype

Grabner and colleagues (Grabner et al., 2013) proposed a neuromuscular healthspan scoring system (NHMSS) constructed from 3 parameters related to muscle strength and endurance (i.e. modelling parts of the Fried operational definition of the frailty syndrome), rotarod performance, grip strength and max isometric force of the extensor digitorum longus muscle. Scores were constructed by averaging the ratio of the parameter to its age group measured mean, as well as to an age-predicted value obtained by a multiple linear regression model using body and heart mass as inputs. However, by definition, construction of this score was only possible for endpoint measurements. Accordingly, predictive power has not been evaluated.

Shortly afterwards, the same group (Liu et al., 2014) proposed a ‘clinically relevant frailty index for mice’. Four of the five Fried criteria were emulated (weakness, slowness, physical inactivity and

exhaustion, but not weight loss) measuring grip strength, walking speed on a rotarod and voluntary wheel running. Exhaustion was emulated by an endurance measure using the averages from grip strength and walking speed measures. Cut-offs were introduced at 1.5 SD below the age-associated mean and frailty was defined as at least 3 out of 4 criteria below cut-off. In this operationalization, exhaustion is clearly a weak criterion due to its interdependence with the grip strength and walking speed measures. To be considered frail, the animal has to match at least three of the defined criteria which are rated in a 5 point scale. With this approach the prevalence of frailty found for cohorts of C56BL/6J mice is 9%, which is consistent with the range of 7–16% calculated by Fried for humans (Fried et al., 2001). Application of this scoring system allowed the discrimination of older mice from unaffected adult animals using a non-invasive approach. However, the validity of this index still needs to be tested in longitudinal and intervention studies as well as in other mouse strains.

2.2. Rockwood-based mouse frailty indices

Parks and colleagues (Parks et al., 2012), who proposed a frailty index according to Rockwood type. In total, 31 parameters were assessed describing physical activity, hemodynamic status, body composition, basic metabolism and organ function. Some measurements were invasive and thus unfavorably harmful for longitudinal follow-up such as drawing high volumes of blood required for metabolic measurements, or repeated exposure to x-rays. Nevertheless a frailty index could be compiled based upon a graded scale measuring deviation from reference averages determined under sex and age-matched conditions. Functional relevance of the index is its distinct association with mouse age, with cardiac hypertrophy and peak contractile force. A frailty index constructed from only 8 non-invasively measured parameters (describing physical activity plus body weight) showed good correlations with the 31-parameter index as well as with cardiomyocyte size. However, its magnitude and variance increased as compared to the 31-item index as expected. Moreover, this study (and especially the functional association studies) was done with very low numbers of animals and, thus, was most probably underpowered.

Later on, the same group went on to propose a different frailty index based exclusively on parameters that can be easily assessed by clinical observation, such as body temperature and weight measurements (Whitehead et al., 2014). As studies in humans had shown decreased discriminatory power of the frailty indices constructed from lower numbers of items, a total 31 parameters were assessed. These items were generally derived from veterinarian practice and used to monitor behavior and distress. Assessment included evaluation of the integument, the musculoskeletal system, auditory, ocular and nasal systems, digestive, urogenital and respiratory systems, signs of discomfort, body weight and body temperature. The severity of each deficit was rated using a simple scale of no deficit, mild or severe grade. In comparison to the 8-item index developed before (Parks et al., 2012), the clinical 31-item index showed improved reproducibility, better discrimination between age groups and predicted mortality (albeit in one single mouse only) and the major advantage of enabling longitudinal assessment. The association of the clinical frailty index with (relative) age in the mouse was very similar to that found between the 70-parameter frailty index and age in humans, as observed in the SHARE study (Börsch-Supan et al., 2013).

3. Validation of frailty scales in mice

In lieu of a fully accepted frailty definition and the little knowledge of its molecular basis, the need for identifying a generally

accepted assessment practice to diagnose and predict frailty is evident. The quantification of frailty in experimental models of ageing, through proper evaluation scales, is a key step to understand the underlying biology of frailty and ageing and to develop meaningful treatments or preventive actions by targeting the involved mechanisms. In this chapter, we will review attempts to validate proposed frailty scales in mice in terms of response to interventions (including genetic interventions) and inter-rater reliability. It should be noted, that assessing the validity and reliability of tools to measure frailty is problematic even for humans where a much larger database is available (Warnier et al., 2016).

Recently, Kane and colleagues (Kane et al., 2016a) analyzed the effects of genetic background (short-lived DBA/2J mice versus long-lived C57Bl/6J) and of interventions with the potential to expand healthspan (40% caloric restriction and treatment with resveratrol) on the Rockwood-type frailty index as proposed by Whitehead et al. (Whitehead et al., 2014). According to this study, both caloric restriction and resveratrol significantly reduced the frailty index in male C57Bl/6J, and there was a tendency for higher frailty scores in the short-lived DBA/2J mice, which however was less than expected from the strain-specific differences in lifespan. This raises the important question to what extent individual components of a frailty index may need to be adjusted to take strain-specific differences into account. In the same study, caloric restriction had no significant effect on the frailty index in females (resveratrol was only tested in males). Other studies have shown strong effects of CR on lifespan in female C57Bl/6 but sex-specific effects on healthspan-related parameters (Cameron et al., 2012). It is possible that the frailty index might be more sensitive to changes in healthspan than in lifespan, but this needs further work to be confirmed or refuted. Frailty-related parameters, but without a full characterization of frailty, were measured in a number of recent intervention studies in male C57Bl/6 mice aimed at expanding healthspan. For instance, Martin-Montalvo et al., 2013 assessed the effect of metformin on spontaneous activity, rotarod and threadmill performance and various metabolic parameters in male mice aged 16–22 months. Xu et al., 2015 measured effects of JAK inhibitor treatment in mice aged around 26 months which were regarded as frail (but not shown to be by any of the proposed scales). The inhibitor improved spontaneous physical activity, grip strength and rotarod performance. Frailty has also been used to stratify cohorts to analyze drug responses in old mice. Kane et al., 2016b found that acetaminophen-induced hepatotoxicity was not different between frail and non-frail old mice, and there was also no change in hepatotoxicity with age overall. Still, stratification by frailty is clearly an important paradigm for intervention studies in old animals and is expected to become more commonly used in the near future.

3.1. Frailty assessment in mutant mouse models of ageing

As for many diseases, genetically modified mice have proven to be invaluable to identify key pathways and mechanistic drivers in the ageing process. This is true for both models of accelerated and delayed ageing. In both cases (albeit with some higher urgency in the case of accelerated ageing models) an essential question is whether the genetic modification targets a fundamental process underlying the whole ageing process in all or at least most of its dimensions or 'only' a specific disease or a 'private' ageing mechanism (Martin-Montalvo et al., 2013), i.e. one of relevance for a single species or strain only. As recently outlined (Richardson et al., 2016), measuring 'healthspan' as an assessment of health over several domains and a wide range of different ages will be necessary to answer the question whether a chosen intervention postpones the ageing process in its entirety or rather, at what costs certain important aspects of ageing will be delayed. However, the much simpler and faster assessment of a treatment-induced shift in frailty should

suffice to provide a first answer to the question posed above, i.e. whether the targeted process has the potential to influence ageing in a fundamental way. Unfortunately, so far there is very little data available on frailty measurements in genetically modified mouse models of ageing.

A wide range of mice models with shortened lifespan and presumably accelerated ageing is available. This includes mice that develop chronic inflammation (*IL-10^{tm/tm}* and *Nfkb1^{-/-}*), mice with accelerated senescence (SAMP), mitochondrial or DNA repair dysregulation (e.g. *Polg^{-/-}*, *Ercc1^{-/-}*, *BubR1* hypomorph), telomerase shortening (*Tert^{-/-}*) and others (Walston et al., 2008; Ko et al., 2011; Sikka et al., 2013; Jurk et al., 2014; Takeda et al., 1981; Derave et al., 2005; Trifunovic et al., 2004; Safdar et al., 2011; Dollé et al., 2006; Baker et al., 2004, 2011; Choudhury et al., 2007).

The relevance of some of these models in ageing research has been questioned, for instance because of their very short lifespan (*Ercc1^{-/-}*) or because they do not resemble any known human condition (SAMP). A formal assessment of a frailty phenotype in these models would thus be useful, but has so far only been performed in mice deficient for the anti-inflammatory cytokine interleukin 10 (*IL-10^{tm/tm}*) according to a frailty scale developed by Walton et al. (Walston et al., 2008) see Table 2). *IL-10^{tm/tm}* mice develop a Crohn's disease-like pathology in colon. However, when *IL10* knockout mice are kept under specific pathogen-free (SPF) conditions, they do not develop colon inflammation but yield a complex phenotype that includes several frailty features, such as sarcopenia, muscular weakness, weight loss as well as age-related increase of serum IL-6, interleukin 1 beta (IL-1 β), tumor necrosis factor alpha (TNF- α), interferon gamma (IFN- γ) and growth-regulated protein alpha (Gro- α /KC), accompanied by blood vessel stiffness and impaired cardiac function (Walston et al., 2008; Ko et al., 2011; Sikka et al., 2013; Sikka et al., 2013). The simultaneous occurrence of all these features in a mouse renders it the first mouse model approximating human frailty syndrome and, therefore, an excellent tool for the study of the interactions between low-grade inflammation, the somatotrophic axis of ageing and the development of frailty. Another genetic model mimicking chronic inflammation is deficiency of NF- κ B p105/p50 subunits in *Nfkb1^{-/-}* mice resulting in a progeroid mouse model in which shorter lifespan and progeria are driven by chronic progressive low grade inflammation. In these mice, a relatively comprehensive assessment of accelerated ageing phenotypes was performed (Jurk et al., 2014) using a range of laboratory parameters in combination with clinical parameters similar to those proposed by Whitehead et al., 2014 for the assessment of frailty. Phenotypes in these mice include, among others, sarcopenia, kyphosis, body weight loss and cardiac hypertrophy. Since the *Nfkb1^{-/-}* mice fulfilled frailty criteria over a wide range of physiological and biological domains Jurk et al., 2014 concluded that progeria in *Nfkb1^{-/-}* mice is not just segmental, thus proving chronic inflammation as a cause of accelerated ageing and frailty. This is in excellent accordance with human studies, in which the chronic activation of inflammatory pathways is closely associated with frailty (Leng et al., 2007) even at extreme old age (Arai et al., 2015) and has long been suspected to play a causal role in its development (Franceschi, 2007). Partial characterizations of frailty/accelerated ageing have been performed in telomerase *Tert^{-/-}* (Choudhury et al., 2007), DNA excision repair cross-complementation group 1 *ERCC1^{-/-}* (Dollé et al., 2006; Niedernhofer et al., 2006), Polymerase gamma (*Polg*) (Trifunovic et al., 2004), BUB1 mitotic checkpoint serine/threonine kinase B (*BubR1*) hypomorphs (Baker et al., 2004) or nucleotide excision repair mice, to name just a few. Of specific interest for a comprehensive assessment of frailty is the senescence-accelerated mouse (SAM) model, which presents several substrains, with a lifespan range between 9.7 and 13.3 months. These mouse lines develop ageing features such as skin lesions, elevated amyloidosis,

Table 2
Comparison of mouse frailty assessment tools.

Liu's Frailty Index ^a	Whitehead's Frailty Index ^b	Walston's Frailty Model ^c
Based on Fried's Frailty Phenotype	Based on Rockwood's Frailty Index	Based on the characterization of IL10 tm genetically modified model
Assesses levels of physical performance and strength/power	Assesses parameters of potential ageing related deficits	Models human frailty state of inflammation, weakness at early age and cardiovascular dysfunction
No Cognitive assessment	Cognitive assessment	Not reported Cognitive assessment
Allows to establish a cut off value for frailty	Quantifies frailty by a well-defined score	Frailty is not quantified but modeled to study its features

^a Ref. Liu et al., 2014.

^b Ref. Whitehead et al., 2014.

^c Ref. Walston et al., 2008.

kyphosis, learning and memory deficits, osteoporosis, muscle loss etc. (Takeda et al., 1981 10; Derave et al., 2005). However, to our knowledge, comparative assessments of frailty in different genetic intervention models have not been done yet. Similarly, there are few data on the effect of pharmacological or lifestyle-type interventions on frailty in mutant ageing mice available, with the notable exception of a study by Wang et al., 2014, testing a nutraceutical grape seed extract in IL-10 knockout mice. Interestingly young mice receiving this extract in drinking water for 12 weeks had enhanced muscle mass, reduced protein degradation and apoptosis, which translated to amelioration of muscle wasting and thus frailty.

3.2. Inter-rater reliability of mouse frailty indices

For frailty assessments to become a useful tool in mouse ageing research, reliability and reproducibility of results between separate mouse cohorts and separate laboratories need to be established. This is especially important as frailty scales are mostly based on rated, rather than measured, parameters. This question has only very recently been addressed, and to our knowledge only for the Rockwood-type index according to Whitehead et al., 2014. Kane et al., 2016b showed that the frailty index developed by Whitehead et al., 2014 gave comparable results to the original publication in a different mouse cohort in a separate laboratory. Moreover, the same paper reported an excellent inter-rater correlation of 0.88 between two raters. In a study by Feridooni et al., 2015 the inter-rater correlation coefficient between two raters was originally lower (around 0.51). However, if individual rater experience was used to change the assessment protocols towards a more clearly defined operationalization, inter-rater correlations increased to values similar to those reported by Kane et al., 2016b. In two subsequent letters published previously (Kane et al., 2015; Howlett and Rockwood, 2015), these results were clarified further. Kane et al., 2015 showed that inter-rater reliability did not necessarily improve just with increasing practice and experience of the raters with the assessment, if it did not result in an adjustment of the assessment protocols. However, raters with different professional background (animal technicians vs scientists) appeared to rate differently, stressing the need for further standardization of the operationalization procedures for the assessment of the frailty index. It is also important to note that the results of Kane et al., 2016b and Feridooni et al., 2015 regarding inter-rater reliability were decidedly different for individual components of the frailty index. Components that were frequently rated different in the Kane et al., 2016b such as vision loss, body condition, grip strength, coat condition, mouse grimace scale, tail stiffening, eye discharge (all with $\leq 80\%$ agreement) were not the same as those rated differently in the Feridooni et al., 2015 which included hearing loss, body temperature, menace reflex, tremor, hunched posture (with $\leq 80\%$ agreement after optimization of protocol).

4. Conclusions: potential for improvement

Frailty is a major phenotype of ageing, and there is an increasing interest to identify and validate interventions than can postpone and/or reverse frailty. Moreover, existing frailty scales and operationalizations are significantly less laborious and expensive to perform as a thorough assessment of healthspan (Richardson et al., 2016). Thus, frailty may be very useful as a simple first indicator and potentially as a screening tool for interventions aimed at improving healthspan in mice. In our opinion, the most important areas for further development to support a wider use are the following:

i) Demonstrate frailty as a useful tool for assessing the efficiency of healthspan-expanding interventions

The limited number of interventions tested so far for their impact on frailty (Kane et al., 2016b; Graber et al., 2015) suggest that frailty assessment is not used as often as it might whereas it is a relatively simple and cheap intervention screening. However, more data will be necessary covering a wider range of interventions. Moreover, data on whether and how well an intervention-mediated change in frailty scores predicts additional ageing phenotypes, especially healthspan and lifespan, will need to be generated. We would like to propose to consider including a frailty score into the phenotypes assessed as part of the NIA Interventions Testing Program (www.nia.nih.gov/research/dab/interventions-testing-program-itp).

ii) Test whether frailty scales are comparable between sexes and amongst mutant strains

There are significant differences in lifespan between males and females from frequently used mouse strains, either under basal conditions or following interventions. Not unfrequently, there seem to be interactions between sex and housing conditions (testing facility) in terms of lifespan. So far, it is not clear whether these sex-related differences in lifespan are reflected in the frailty scores of the mice. In genetically modified mice with either a shortened or extended lifespan, formal frailty assessments have almost never been performed (with the notable exception of the IL-10^{tm/tm} mice (Walston et al., 2008)), although some of the components of Fried- and Rockwood-type frailty scales have been employed as measures of healthspan in these mice. There is a concern that some components of frailty scales (for instance hearing loss or cataract) are expressed in a strongly strain-specific fashion, which would complicate the assessment of a mutant in comparison to wild-type littermates. However, strain-specific normalization of the scales is a possibility to overcome this problem.

iii) Optimise the frailty concept, validate different frailty scales against each other

Presently, at least three different concepts have been used to measure frailty in mice (Walston et al., 2006; Whitehead et al., 2014; Liu et al., 2014). It is not clear how well the concordance between these concepts is. In humans, Fried and Rockwood frailty assessments overlap significantly, but not completely. Interest-

ingly, both scales showed largely consistent associations providing a range of candidate biomarkers for ageing in a very old population (Collerton et al., 2012).

iv) Develop the operationalization protocols and clarify the extent of reproducibility

In terms of frailty scale operationalization and reproducibility, the situation is different for the Fried- and Rockwood-type assays. To our knowledge, no data are available yet about the reproducibility of the Fried-type frailty assessments (assay according to Liu et al., 2014) in mice between observers and/or laboratories. It might be assumed, that this assay is less sensitive to inter-observer variation, because fewer parameters are taken into account and these are measured rather than rated. As discussed above, we believe that interdependency of the parameters used for operationalization of frailty by Liu et al. is a weakness of the index. We suggest that inclusion of a measure of weight loss might improve the assay. Unintentional weight loss is a criterion in the human Fried frailty scale and weight loss is a strong predictor of increased mortality rate in humans (Miller and Wolfe, 2008). Mice (under ad libitum feeding) also show a steady decline of body weight during their final weeks to months of life, and the age at which their body weight reached its maximum was predictive of lifespan and associated with other healthspan measures in a study testing the role of chronic inflammation in mice (Jurk et al., 2014).

By design, the Rockwood deficit scale is open for the incorporation of additional parameters. A biomarker frailty index has already been constructed for humans (Mitnitski et al., 2015). For mice, age-associated changes in the vasculature, such as increased central elastic large artery stiffness and endothelial dysfunction assessed by pulse wave velocity could be informative, as could be markers of bone degeneration and osteoporosis or inflammation. There have been discussions whether and to which extent organ-specific lesion markers should be included into frailty scales of the Rockwood type. There are clearly multiple lesions that develop with age across a wide range of organs including lungs, kidneys, bladder, heart, spleen, adrenal glands, liver and others (Rowlatt et al., 1976; Turturro et al., 2002). However, if one accepts low cost and ease of operation as major advantages of mouse frailty assessments, it appears that the index according to Whitehead et al. already provides a good compromise between wide-ranging coverage of ageing phenotypes and operational cost. As this index is largely based on observation scores rather than objective measurements, inter-rater reliability is an important issue. The few available data are very encouraging, as generally high correlation coefficients were obtained when two independent ratings were compared. At the same time, these results indicate the importance of developing operating protocols as detailed and clearly as possible. Furthermore, the dependency of ratings on professional background needs further interrogation and ways to operationalize the assessment such that background bias is minimized need to be found.

In conclusion, a broad consensus exists that frailty entails multi-organ dysfunction and increased vulnerability to additional diseases, which suggests a link between mortality and frailty. Means and measures for frailty assessment in combination with a panel of valid biomarkers, which remain to be identified and standardized, will allow diagnosis and follow up of frailty conditions.

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